Protective effects of ursodeoxycholic acid in experimental corrosive esophagitis injury in rats

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Accidental caustic ingestions are serious medical problems especially in childhood. Various treatment modalities are being used for the complications of caustic injuries such as stricture formation. The aim of this study is to establish whether ursodeoxycholic acid (UDCA) has protective effects on experimental corrosive esophagitis in rats.
Twenty four Wistar-albino rats, weighing 220-240 g, were used in the study. Experimental animals were divided in three groups randomly: UDCA treatment group (Group T, n:8), control group (Group K, n: 8) and sham group (Group S, n: 8). In group T and S corrosive esophagitis was induced. UDCA (5 mg/kg) was performed to the group T for 10 days orally. All animals were sacrificed at the end of procedures and histopathological changes in esophageal tissue were scored by a single investigator who was blind to the groups.
In group T inflammation was present in two rats, muscularis mucosa injury in two rats, grade 1 collagen deposition in six rats and grade 2 in two rats. In comparison with group S these were statistically significant (p value was 0.003, 0.003 and 0.015, respectively).
UDCA has protective effect in experimental corrosive esophagitis.

KEY WORDS: Corrosive esophagitis, Rat, Stricture, Ursodeoxycholic acid

Introduction

Caustic and corrosive ingestion causes upper gastrointestinal tract injuries especially in esophagus from superficial erosion to stricture formation, perforation and rarely death [1]. Particularly, caustic ingestion causes serious medical problems in childhood and 20% of the cases have stricture formation in time. Steroids, antibiotics, and esophageal dilations are preferred initial treatment options of caustic esophageal injury complications. However, there are some controversies about the treatment method to be used and the duration. Also none of all these methods was effective in serious injuries [2-4]. The main histopathologic changes of esophageal caustic burn are collagen deposition, remodeling, esophagus wall thickness and dense connective tissue formation. So, inhi-

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bition of inflammatory reactions and the stricture formation are the aim of medical treatment of caustic esophageal burns, and various treatment methods are used to prevent fibrosis formation [5]. Complications are closely related with severity of caustic injury and the initial esophageal damage. For that reason, initial treatment is very important in this kind of injuries [6].

Ursodeoxycholic acid (UDCA) is produced from epimerization of 7 β chenodeoxycholic acid by intestinal bacteria and composed of 1-3% of bile acid pool [7]. Recently, Ursodeoxycholic acid is used for treatment of many liver diseases with or without cholestasis. Mechanism of actions of UDCA can be summarized as displacement of toxic endogenous bile acid, cytoprotective effect, cell membrane protection, stabilization, antiapoptotic effects, immunomodulatory effect, formation of bile duct epithelium, stimulation of bile secretion from hepatocytes, stimulation of exocytosis, location of canalicular membrane transporters and stimulation of defective gene expression of hepatobiliary transport systems [8].

Although a lot of protective materials have been used for corrosive esophageal burns, UDCA has not been studied in literature. The aim of this study is to establish whether UDCA has mucosal protective effects on experimental corrosive esophagitis in rats.

Material and Methods

The study was done in Çanakkale Onsekiz Mart University (ÇOMU) Experimental Animal Center after animal care and all procedures were approved by the Animal Care Committee of ÇOMU

Experimental Groups

Twenty four Wistar-albino rats, weighing 220-240 g, were used in the study. Experimental animals were divided in three groups randomly: UDCA treatment group (Group T, n: 8), control group (Group K, n: 8) and sham group (Group S, n:8).

Experimental Model

Before the caustic burn performed the rats had been fastened during 12 hours and ketamine 50 mg/kg and xylazine 5 mg/kg intraperitoneally (IP) were used for general anesthesia in all three groups. The median laparotomy incision technic was used to open the abdominal cavity and gastroesophageal junction was suspended with 3.0 silk in group T and K. Then, esophagus was suspended one cm proximal of gastroesophageal junction with 3.0 silk. This separated esophageal segment was exposed to 0,1 ml 37.5% NaOH (pH=12) for 10 seconds. After 20 sec of releasing first suspender the second one was released and the abdomen was closed. Afterwards all experimental rats were fed by water and rat food. UDCA (5 mg/kg) was performed to the group T for 10 days orally.

Histopathologic Evaluation

Esophagus was removed two weeks later under general anesthesia, fixed in 10% neutral formalin solution and then embedded in paraffin and stained with hemotoxylin-eosin, all rats. 4-μm thick sections were examined under a light microscope by a histopathologist. Histopothologic evaluation was performed in all experimental rats and then findings were scored according to muscularis mucosal damage, collagen deposition of tunica muscularis and submucosal collogen increase [9] (Table I).

Statistical Analysis

For the comparison of nonparametric data Kruskal–Wallis test was used. $P < 0.05$ was considered statistically significant.

Table I - *Histopathological results of Groups*

Histopathological finding	Group K	Group S	Group T	p*
Inflammation	0/+ 8/-	8/+ 0/-	2/+ 6/-	<0,001
Injury in muscularis mucosa	0/+ 8/-	8/+ 0/-	2/+ 6/-	<0,001
Collagen deposition in tunica muscularis				
Grade 0	8/+	0/+	0/+	<0,001
Grade 1		1/+	6/+	
Grade 2		7/+	2/+	

*Kruskal Wallis Test

Results

In histopathologic examination of group K there was no inflammation, muscularis mucosa injury or collagen deposition in tunica muscularis. In group S, there was inflammation and muscularis mucosa injury in all rats, collagen deposition in tunica muscularis was grade 1 in on erat and grade 2 in seven rats.
In group T inflammation was present in two rats, muscularis mucosa injury in two rats , grade 1 collagen deposition in six rats and grade 2 in two rats. In comparison with group S these were statistically significant (p value was 0.003, 0.003 and 0.015, respectively). In group T, all injuries decrease significantly. The results of histopathologic examinations are summarized in table I. Histopathologic specimens of groups were shown in Figs. 1, 2.

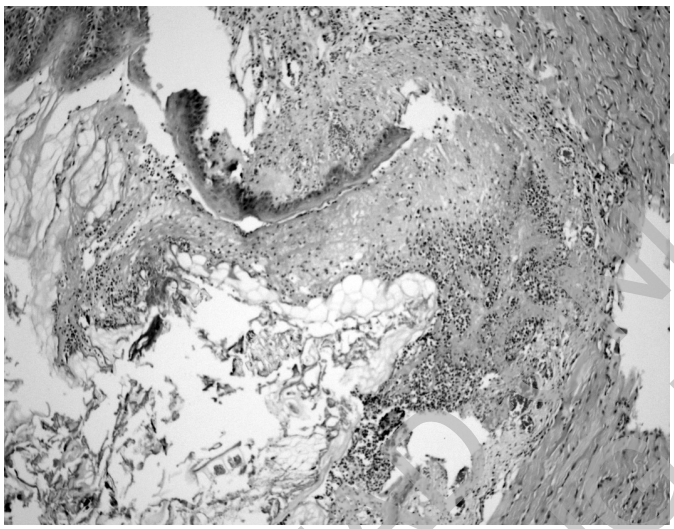

Fig. 1: Group S: Severe inflammation with polymorphonuclear infiltration, more severe inflammation with ulcers (arrows) (H&E 100X).

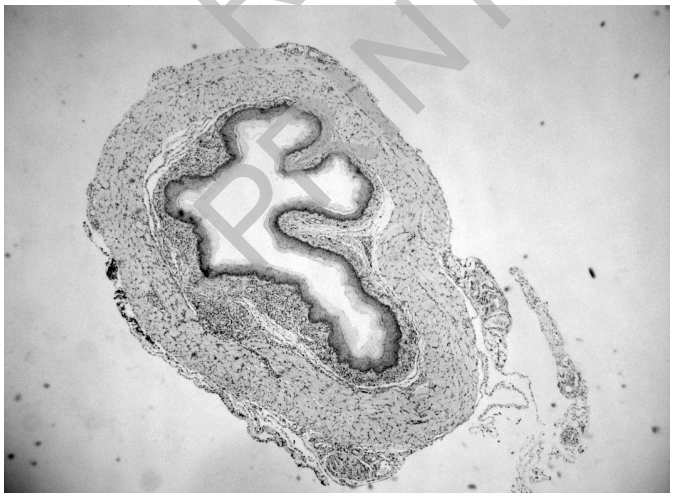

Fig. 2: Group T: Minimal inflammatory changes on the 10. Days in treated rats. (H&E 40X).

Discussion

UDCA, used commonly in hepatic and common biliary track diseases but not for esophagus, was effective in the treatment of corrosive esophageal burns in this experimental study.
Corrosive substances can harm both histologically and functionally in case of ingestion 10. After corrosive contact mucosal damage occurs within seconds and deep tissue damage arises following minutes. Concentration, volume and pH of corrosive substance, location of corrosive burn and the duration are the important factors of severity of tissue injuries [11, 12]. It was shown that one second tissue exposure of 30 % NaOH could cause full-thickness necrosis at experimental animals 13. In our study, esophageal tissues of rats were exposed to 37,5 % NaOH for 10 sec.
In the first week of caustic injury acute inflammatory response arises. Beginning of fibroblastic proliferation and collagen formation can be seen in the second week [14].
It is known that, alkaline agent that we used in our study has three-stage damage [15, 16]. The first stage is acute necrotic phase which lasts 1-4 days and coagulation of intracellular proteins and cellular necrosis take place. The second phase, peeling of superficial necrotic tissue and ulceration, begins at 3-5 days after exposure and lasts for 10-12 days. The resulting defect is filled with granulation tissue. The last one, third week, is scar formation stage. Due to contraction of connective tissue and scar formation, esophageal narrowing occurs.
The degree of corrosive esophageal burns varies from superficial mucosal hyperemia and edema to ulceration, full-thickness necrosis and deep ulcers that may cause tracheoesophageal fistula (17). The most serious complication of corrosive ingestion is stricture formation, seen in 5-20% of the victims and lasts lifelong [2, 18, 19]. In order to prevent these harmful effects, esophagus and salivary glands secretes some protective substances such as bicarbonate, mucus, PGE 2, TGF-α and epidermal growth factor [20].
The most important factors affecting the prognosis are early diagnosis and treatment. Esophagoscopy is a valuable, fast and effective method to determine the extent and severity of the tissue damage [21]. However, there is no complete consensus on exact treatment options and the duration of the treatment. In addition to the own physiological protective barriers of esophagus , there are some treatment options of corrosive esophagitis such as ; antibiotics , corticosteroids with or without antibiotics, total parenteral nutrition, nasogastric tubes, stent placement into the lumen of the esophagus, dilatation of esophagus with balloon or combination of these treatment methods .
It is known that when used in the treatment of gastroesophageal reflux UDCA reduces the adverse effects of reflux material. In addition to anti-inflammatory and immunomodulatory effects, UDCA has regulatory effects

on gastrointestinal motility and prevents duodeno-gastric reflux. It is documented that UDCA protects cells from apoptosis by activating glucocorticoid and mineralocorticoid receptors [22]. A double –blind study by Realini et al. [23], 150 mg UDCA, applied twice a day for 14 days in the treatment of dyspeptic complaints, showed better effect when compared to placebo group. In another study 12 reflux gastritis patients were treated with UDCA, despite recovery of clinical symptoms, histological improvement could not be demonstrated [24]. Although our study was not a gastroesophageal reflux model, significant histologic improvement was shown in treated group of corrosive esophagitis with UDCA.

In conclusion, UDCA may be used in the treatment of corrosive esophagitis, but comprehensive studies are needed to understand dose range and treatment duration.

Riassunto

L'ingestione accidentale di caustici è un problema serio di competenza medica soprattutto nell'infanzia. Vengono utilizzate diverse modalità di trattamento per le complicazioni delle lesioni da caustici, come la formazione di stenosi. Lo scopo di questo studio è quello di stabilire se l'acido ursodesossicolico (UDCA) ha effetti protettivi per le mucose e impedisce la formazione di stenosi nell'esofagite corrosiva sperimentale nei ratti.

Nello studio sono stati utilizzati ventiquattro ratti Wistar-albini, peso tra 220-240 g. Gli animali da esperimento sono stati divisi in tre gruppi in modo casuale: un gruppo trattato con UDCA (gruppo T, n: 8), un gruppo di controllo (gruppo K, n: 8) e il gruppo sham (Gruppo S , n: 8). L' esofagite corrosiva è stata indotta nei gruppi T e S. Al gruppo T è stato somministrato UDCA (5 mg/kg) per via orale per 10 giorni. Tutti gli animali sono stati sacrificati al termine della procedura e i cambiamenti istopatologici del tessuto esofageo sono stati valutati, in cieco, da un singolo ricercatore che era all'oscuro dei gruppi.

Nel gruppo T è stata riscontrata infiammazione in due ratti, lesioni alla muscolaris mucose in due ratti, deposizione di collagene di grado 1 in sei ratti e di grado 2 in due ratti. In confronto con il gruppo S questi erano statisticamente significative (p value è 0,003, 0,003 e 0,015, rispettivamente).

UDCA ha un effetto protettivo nell'esofagite corrosiva sperimentale.

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